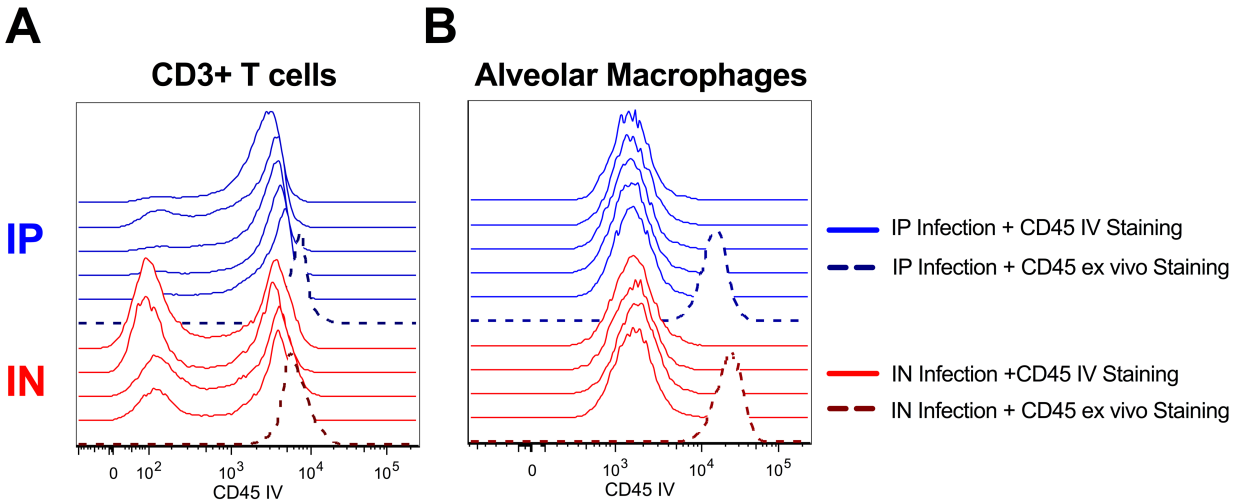
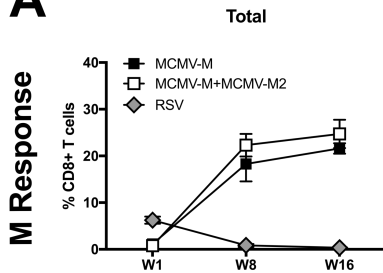


Supplementary Data

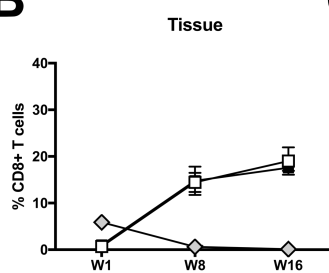


Supplementary Figure 1. Intravascular staining is not affected by Intranasal vaccination with MCMV. Mice were vaccinated with MCMV-M via the IN (red) or IP (blue) route. On day 7 post-vaccination, mice were injected IV with 3 μ g of anti-CD45-PerCP-Cy5.5 and euthanized 5 minutes later. Lungs were harvested and stained ex vivo for (A) T cells (CD3+) and (B) alveolar macrophages (CD3-CD11c⁺CD64⁺SiglecF⁺). Lines represent individual mice with 4-5 mice/group. Lungs from one mouse/group were stained ex vivo for CD45 (dashed lines).

A

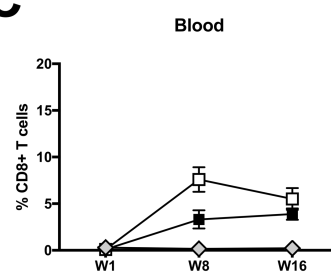
	W1 vs W8	W1 vs W16	W8 vs W16
MCMV-M	****	****	NS
MCMV-M+MCMV-M2	****	****	NS
RSV	NS	NS	NS

	W1	W8	W16
MCMV-M vs MCMV-M+MCMV-M2	NS	NS	NS
MCMV-M vs RSV	NS	****	****
MCMV-M+MCMV-M2 vs RSV	NS	****	****

B

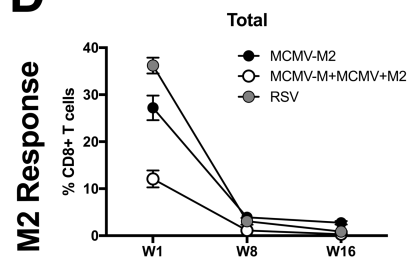
	W1 vs W8	W1 vs W16	W8 vs W16
MCMV-M	****	****	NS
MCMV-M+MCMV-M2	****	****	NS
RSV	NS	*	NS

	W1	W8	W16
MCMV-M vs MCMV-M+MCMV-M2	NS	NS	NS
MCMV-M vs RSV	NS	****	****
MCMV-M+MCMV-M2 vs RSV	NS	****	****

C

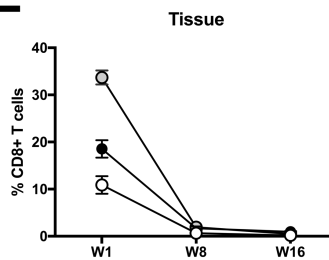
	W1 vs W8	W1 vs W16	W8 vs W16
MCMV-M	**	**	NS
MCMV-M+MCMV-M2	****	****	NS
RSV	NS	NS	NS

	W1	W8	W16
MCMV-M vs MCMV-M+MCMV-M2	NS	***	NS
MCMV-M vs RSV	NS	**	**
MCMV-M+MCMV-M2 vs RSV	NS	****	****

D

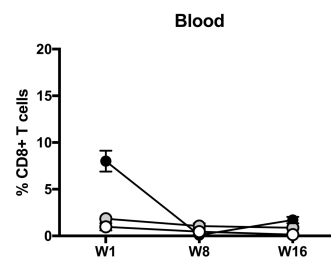
	W1 vs W8	W1 vs W16	W8 vs W16
MCMV-M2	****	****	NS
MCMV-M+MCMV-M2	****	****	NS
RSV	****	****	NS

	W1	W8	W16
MCMV-M2 vs MCMV-M+MCMV-M2	****	NS	NS
MCMV-M2 vs RSV	****	NS	NS
MCMV-M+MCMV-M2 vs RSV	****	NS	NS

E

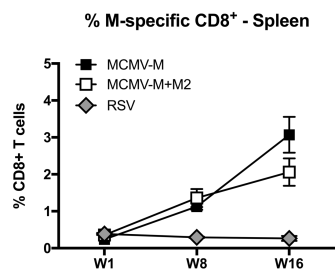
	W1 vs W8	W1 vs W16	W8 vs W16
MCMV-M2	****	****	NS
MCMV-M+MCMV-M2	****	****	NS
RSV	****	****	NS

	W1	W8	W16
MCMV-M2 vs MCMV-M+MCMV-M2	****	NS	NS
MCMV-M2 vs RSV	****	NS	NS
MCMV-M+MCMV-M2 vs RSV	****	NS	NS

F

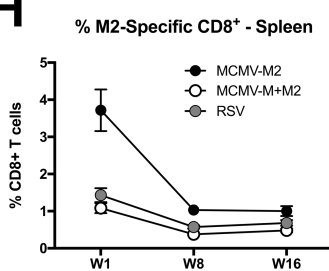
	W1 vs W8	W1 vs W16	W8 vs W16
MCMV-M2	****	****	*
MCMV-M+MCMV-M2	NS	NS	NS
RSV	NS	NS	NS

	W1	W8	W16
MCMV-M2 vs MCMV-M+MCMV-M2	****	NS	NS
MCMV-M2 vs RSV	****	NS	NS
MCMV-M+MCMV-M2 vs RSV	NS	NS	NS

G

	W1 vs W8	W1 vs W16	W8 vs W16
MCMV-M	*	****	****
MCMV-M+MCMV-M2	**	****	NS
RSV	NS	NS	NS

	W1	W8	W16
MCMV-M vs MCMV-M+MCMV-M2	NS	NS	NS
MCMV-M vs RSV	NS	*	**
MCMV-M+MCMV-M2 vs RSV	**	****	****

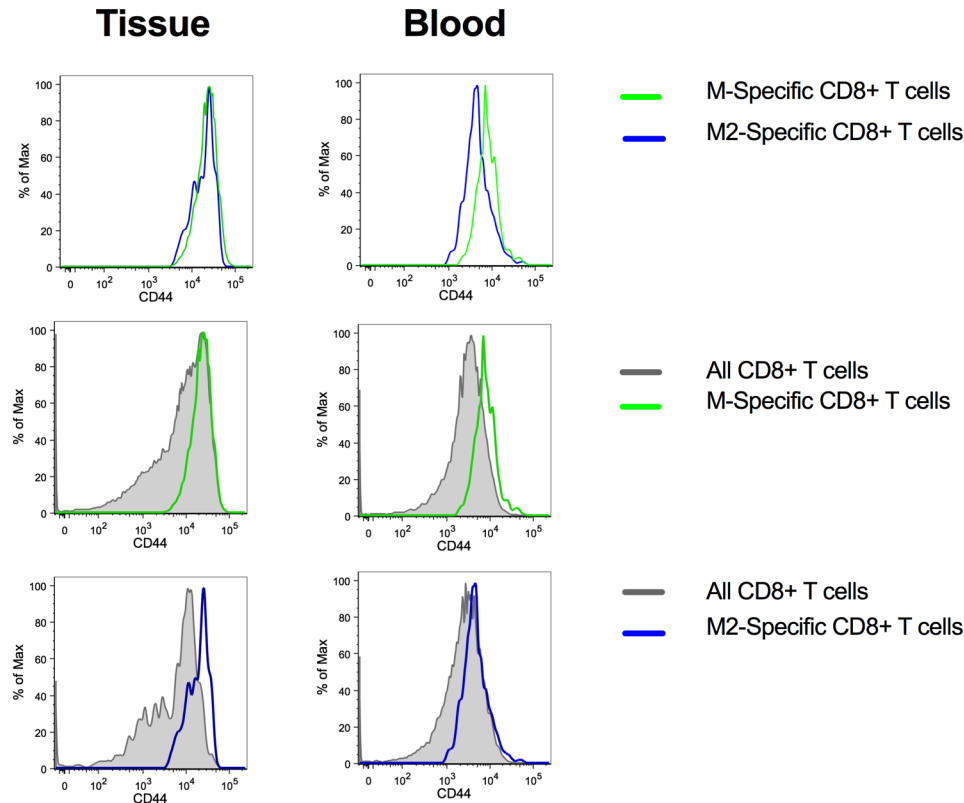
H

	W1 vs W8	W1 vs W16	W8 vs W16
MCMV-M2	****	****	NS
MCMV-M+MCMV-M2	NS	NS	NS
RSV	*	NS	NS

	W1	W8	W16
MCMV-M2 vs MCMV-M+MCMV-M2	****	NS	NS
MCMV-M2 vs RSV	****	NS	NS
MCMV-M+MCMV-M2 vs RSV	NS	NS	NS

Supplementary Figure 2. The M-specific CD8⁺ T cell population inflates, whereas the M2-specific CD8⁺ T cell population contracts, after vaccination with MCMV.

(A–F) Mice were infected with RSV or vaccinated with MCMV-M or MCMV-M2 alone or a combination of MCMV-M and MCMV-M2 via the intranasal route. Intravascular staining was used in conjunction with D^b/M₁₈₇₋₁₉₅ and K^d/M2₈₂₋₉₀ tetramers to determine the frequency of M-specific (A–C) and M2-specific (D–F) CD8⁺ T cells in the lung tissue and blood at weeks 1 (W1), 8 (W8), and 16 (W16). Total (A, D) denotes all tetramer⁺ CD8⁺ T cells regardless of location. (G,H) Frequency of total M-specific (G) and M2-specific (H) CD8⁺ T cells in the spleen. Bars indicate mean ± SEM (n = 5 mice/group). **** P < 0.0001, *** P < 0.001, ** P < 0.01, * P < 0.05 by two-way ANOVA. Data shown from one experiment and representative of two independent experiments.



Supplementary Figure 3. Comparison of CD44 expression on M-specific and M2-specific CD8⁺ T cells. CD44 expression was determined on M-specific (green), M2-specific (blue), and all (grey shaded) CD8⁺ T cells at week 8 after vaccination with MCMV-M or MCMV-M2. Intravascular staining was used in conjunction with D^b/M₁₈₇₋₁₉₅ and K^d/M₂₈₂₋₉₀ tetramers to identify M-specific and M2-specific CD8⁺ T cells in the blood and tissue of the lungs. Data are representative two independent experiments (n = 5 mice/group).